

# THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

Utah State University

MICEUS, THERE HAS BEEN PRESENTED TO THE

# Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROJECTION FOR AN ALLEGED DISTINCT VARIETY OF SEXUALLY REPRODUCED, OR TUBER PROPAGATED PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLIED WITH, AND THE TITLE THERETO IS, FROM THE RECORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID COPY, AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

NOW, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT UNTO THE SAID APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT(S) FOR THE TERM OF TWENTY YEARS FROM THE DATE OF THIS GRANT, SUBJECT TO THE PAYMENT OF THE REQUIRED FEES AND PERIODIC REPLENISHMENT OF VIABLE BASIC SEED OF THE VARIETY IN A PUBLIC REPOSITORY AS PROVIDED BY LAW, THE TIGHT TO EXCLUDE OTHERS FROM SELLING THE VARIETY, OR OFFERING IT FOR SALE, OR REPRODUCING IT, OR PORTING IT, OR EXPORTING IT, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE VE PURPOSES, OR USING IT IN PRODUCING A HYBRID OR DIFFERENT VARIETY THEREFROM, TO THE EXTENT BE SOLD BY VARIETY PROTECTION ACT. IN THE UNITED STATES SEED OF THIS VARIETY BE SOLD BY VARIETY NAME ONLY AS A CLASS OF CERTIFIED SEED AND (2) SHALL CONFORM TO THE NUMBER OF THE RIGHTS. (84 STAT. 1542, AS AMENDED, 7: U.S.C. 2321

### BARLEY

'Aquila'

In Jestimon Wherest, I have hereunto set my hand and caused the seal of the Plant Inriety Protection Office to be affixed at the City of Washington, D.C. this sixth day of February, in the year two thousand and seven.

Attest:

ET SÎ

20 m Jula

Commissioner Plant Variety Protection Office Saricultural Warketina Service ry of Agriculture

REPRODUCE LOCALLY. Include form number and o			<u> </u>				
U.S. DEPARTMENT OF AGRICULTURE AGRICULTURAL MARKETING SERVICE SCIENCE AND TECHNOLOGY - PLANT VARIETY PROTECTION OFFICE  APPLICATION FOR PLANT VARIETY PROTECTION CERTIFICATE (Instructions and information collection burden statement on reverse)			the I	The following statements are made in accordance with the Privacy Act of 1974 (5 U.S.C. the Paperwork Reduction Act (PRA) of 1995.  Application is required in order to determine if a plant variety protection certificate is to be (7 U.S.C. 2421). Information is held confidential until certificate is issued (7 U.S.C. 2426).			
			Appli (7 U.				
1. NAME OF OWNER				EMPORARY DESIGNATION O XPERIMENTAL NAME	R 3. VA	RIETY NAME	
Utah State University			τ	JT95B1480-1632	Aqu	ila	
ADDRESS (Street and No., or R.F.D. No., City,	State, and ZIP Co	de, and Country)	5, T	ELEPHONE (include area code	)	FOR OFFICIAL USE ONLY	
4820 Old Main Hill			(4	(435) 797-7214 PVPO NUMBER			
Logan, UT 84322				AX (include area code)	- 2	0060004	
			(43	35) 797-3376		G DATE	
. IF THE OWNER NAMED IS NOT A "PERSON",		8. IF INCORPORATED, GIVE		ATE OF INCORPORATION			
ORGANIZATION (corporation, partnership, asso	ociation, etc.)	STATE OF INCORPORATION	`			11-29-2005	
0. NAME AND ADDRESS OF OWNER REPRES	ENTATIVE(S) TO	REDIVE IN THIS ADDITIONAL (SIX	et nareon li	etod will monive all nanomi	<u> </u>	FILING AND EXAMINATION FEES:	
O. MAINE AND ADDRESS OF OWNER REFRES	ENTATIVE(S) TO	SERVE IN THIS APPLICATION, (FIIS	si person ii	sted will receive all papers)	É		
Dr. Dominique Roche					š	s 3652	
Plants, Soils, & Biometeorology	Dept.				R E	DATE 11/29/05	
Utah State University					E C	CERTIFICATION FEE:	
Logan, UT 84322-4820					Į į	\$ 768,00	
					E	DATE 10/24/200	
TELEPHONE (Include area code)	12. FAX (Includ	de area code)	-	13. E-MAIL		1 7-11-100	
(435) 797-7214	(435) 797	7-3376	d	lroche@mendel.usu.ed	u		
4. CROP KIND (Common Name)	16. FAMILY N	AME (Botanical)		18. DOES THE VARIETY CONTAIN ANY TRANSGENES? (OPTIONAL)			
Barley	Poaceae (Gr	ramineae)		YES 🗹 NO			
5. GENUS AND SPECIES NAME OF CROP	17. IS THE VAR	RIETY A FIRST GENERATION HYBR	RID?			O USDA-APHIS REFERENCE NUMBER	
Fordeum vulgare	□YES	<b>☑</b> NO		COMMERICALIZATION.		LATE THE GENETICALLY MODIFIED PI	
CHECK APPROPRIATE BOX FOR EACH ATT     (Follow instructions on reverse)	I ACHMENT SUBMI	TTED	7	20. DOES THE OWNER SPEC OF CERTIFIED SEED? (3	OIFY THAT S	BEED OF THIS VARIETY BE SOLD AS A 83(a) of the Plant Variety Protection Act)	
a. Exhibit A. Origin and Breeding History	of the Variety			YES (If "yes", answ			
b.  Exhibit B. Statement of Distinctness			2	21. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS T NUMBER OF CLASSES?			
c.  Exhibit C. Objective Description of Va.	riety			YES NO	)		
d.  Exhibit D. Additional Description of the	-					INDATION ☐ REGISTERED ☐ CE	
hearest .			2	22. DOES THE OWNER SPEC	IFY THAT S	SEED OF THIS VARIETY BE LIMITED AS	
e. Exhibit E. Statement of the Basis of the				NUMBER OF GENERATIONS?			
<ol> <li>Voucher Sample (2,500 viable untreative infication that tissue culture will be direpository)</li> </ol>				YES INO  IF YES, SPECIFY THE NUMBER 1,2,3, etc. FOR EACH CLASS.			
g. Filing and Examination Fee (\$3,652), r	nade payable to "T	reasurer of the United					
States" (Mail to the Plant Variety Prote	ction Office)			FOUNDATION REGISTERED CERTIFIED  (If additional explanation is necessary, please use the space indicated on the rever			
HAS THE VARIETY (INCLUDING ANY HARVE FROM THIS VARIETY BEEN SOLD, DISPOSE OTHER COUNTRIES?			7	24. IS THE VARIETY OR ANY COMPONENT OF THE VARIETY PROTECTED BY INTELLECTUAL PROPERTY RIGHT (PLANT BREEDER'S RIGHT OR PATENT,			
YES I NO				TYES INO			
IF YES, YOU MUST PROVIDE THE DATE OF FIRST SALE, DISPOSITION, TRANSFER, OR USE FOR EACH COUNTRY AND THE CIRCUMSTANCES. (Please use space indicated on reverse.)				IF YES, PLEASE GIVE COU REFERENCE NUMBER. <i>(F</i>		TE OF FILING OR ISSUANCE AND ASSI pace indicated on reverse.)	
The owners declare that a viable sample of bas a tuber propagated variety a tissue culture will it	sic seed of the varie	ety has been furnished with application	on and will he duration	be replenished upon request in not the certificate.	accordance	with such regulations as may be applic	
The undersigned owner(s) is(are) the owner of	this sexually reprod	duced or tuber propagated plant varie	ety, and be	lieve(s) that the variety is new,	distinct, unif	orm, and stable as required in Section 4:	
entitled to protection under the provisions of Se	ction 42 of the Plan	nt Variety Protection Act,					
Owner(s) is (are) informed that false represents	ation herein can jeo	pardize protection and result in pena	Ilties.	1.	,	/ 1	
SNATURE OF OWNER	······································	***************************************	SIGNATUR	REOFOWNER	/		
Haul Samurs	in		NAME /O/-	pase print or type)	. <i>L</i>	ub	
MAUL KACIALISSEL	/		NAME (PIE	TOWN J.	J.	KyB/fen	
PACITY OR TITLE	DATE	1-20-05	CAPACITY	OR TITLE	DATE	11/22/2	
CICYC IST M. Sant. )	ΓC.   11	1-20-03	-UI			11/ / 0	
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INSTRUCTIONS

200600040

GENERAL: To be effectively filed with the Plant Variety Protection Office (PVPO), ALL of the following items must be received in the PVPO: (1) Completed application form signed by the owner; (2) completed exhibits A, B, C, E; (3) for a seed reproduced variety at least 2,500 viable untreated seeds, for a hybrid variety at least 2,500 untreated seeds of each line necessary to reproduce the variety, or for tuber reproduced varieties verification that a viable (in the sense that it will reproduce an entire plant) tissue culture will be deposited and maintained in an approved public repository; (4) check drawn on a U.S. bank for \$3,652 (\$432 filing fee and \$3,220 examination fee), payable to "Treasurer of the United States" (See Section 97.6 of the Regulations and Rules of Practice.) Partial applications will be held in the PVPO for not more than 90 days, then returned to the applicant as unfilled. Mali application and other requirements to Plant Variety Protection Office, AMS, USDA, Room 401, NAL Building, 10301 Baltimore Avenue, Beltsville, MD 20705-2351. Retain one copy for your files. All items on the face of the application are self explanatory unless noted below. Corrections on the application form and exhibits must be initialed and dated. DO NOT use masking materials to make corrections. If a certificate is allowed, you will be requested to send a check payable to "Treasurer of the United States" in the amount of \$432 for issuance of the certificate. Certificates will be issued to owner, not licensee or agent.

Plant Variety Protection Office Telephone: (301) 504-5518 FAX: (301) 504-5291

Homepage: http://www.ams.usda.gov/science/pvpo/pvpindex.htm

To avoid conflict with other variety names in use, the applicant must check the appropriate recognized authority and provide evidence that name has been cleared by the appropriate recognized authority before the Certificate of Protection is issued. For example, for agricultural and vegetable crops, contact: Seed Branch, AMS, USDA, 10301 Baltimore Avenue, Suite 401 NAL Building, Beltsville, MD 20705. Telephone: (301) 504-5682 http://www.ams.usda.gov/lsg/seed.htm.

#### ITEM

- 19a. Give:
- (1) the genealogy, including public and commercial varieties, lines, or clones used, and the breeding method;
- (2) the details of subsequent stages of selection and multiplication;
- (3) evidence of uniformity and stability; and
- (4) the type and frequency of variants during reproduction and multiplication and state how these variants may be identified
- 19b. Give a summary of the variety's distinctness. Clearly state how this application variety may be distinguished from all other varieties in the same crop. If the new variety is most similar to one variety or a group of related varieties:
  - (1) identify these varieties and state all differences objectively;
  - (2) attach statistical data for characters expressed numerically and demonstrate that these are clear differences; and
  - (3) submit, if helpful, seed and plant specimens or photographs (prints) of seed and plant comparisons which clearly indicate distinctness.
- 19c. Exhibit C forms are available from the PVPO Office for most crops; specify crop kind. Fill in Exhibit C (Objective Description of Variety) form as completely as possible to describe your variety.
- 19d. Optional additional characteristics and/or photographs. Describe any additional characteristics that cannot be accurately conveyed in Exhibit C. Use comparative varieties as is necessary to reveal more accurately the characteristics that are difficult to describe, such as plant habit, plant color, disease resistance, etc.
- 19e. Section 52(5) of the Act requires applicants to furnish a statement of the basis of the applicant's ownership. An Exhibit E form is available from the PVPO.
- 20. If "Yes" is specified (seed of this variety be sold by variety name only, as a class of certified seed), the applicant MAY NOT reverse this affirmative decision after the variety has been sold and so labeled, the decision published, or the certificate issued. However, if "No" has been specified, the applicant may change the choice. (See Regulations and Rules of Practice, Section 97.103).
- 23. See Sections 41, 42, and 43 of the Act and Section 97.5 of the regulations for eligibility requirements.
- 24. See Section 55 of the Act for instructions on claiming the benefit of an earlier filing date.
- 22. CONTINUED FROM FRONT (Please provide a statement as to the limitation and sequence of generations that may be certified.)
- 23. CONTINUED FROM FRONT (Please provide the date of first sale, disposition, transfer, or use for each country and the circumstances, if the variety (including any harvested material) or a hybrid produced from this variety has been sold, disposed of, transferred, or used in the U.S. or other countries.)
- 24. CONTINUED FROM FRONT (Please give the country, date of filing or issuance, and assigned reference number, if the variety or any component of the variety is protected by intellectual property right (Plant Breeder's Right or Patent).)

NOTES: It is the responsibility of the applicant/owner to keep the PVPO informed of any changes of address or change of ownership or assignment or owner's representative during the life of the application/certificate. The fees for filing a change of address; owner's representative; ownership or assignment; or any modification of owner's name is specified in Section 97.175 of the regulations. (See Section 101 of the Act, and Sections 97.130, 97.131, 97.175(h) of the Regulations and Rules of Practice.)

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0581-0055. The time required to complete this information collection is estimated to average 1.4 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, gender, religion, age, disability, sexual orientation, marital or family status, political beliefs, parental status, or protected genetic information. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braitle, large print, audiotape, etc.) should contact USDA's TARGET Center at 202-720-2600 (voice and TDD).

To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 14th and Independence Avenue, SW, Washington, DC 20250-9410 or call 202-720-5964 (voice and TDD). USDA is an equal opportunity provider and employer.

# Exhibit A - Origin and Breeding History

## **AQUILA**

Summer, 1992:

Original cross made at Logan, Utah, by Dr. Rulon S. Albrechtsen Cross number was UT97B 1480

UT-S.D.B1-1009/M72-395/3/Utah Short#2// ID633019/'Woodvale'/4/'Steptoe'/M27//'Westbred Gustoe'

UT-S.D.B1-1009 is a sib to Bracken (Albrechtsen, *Crop Sci.*33: 1413-1414; 1993).

M72-395 is a short stature breeding line involving the parents 'Jotun', 'Kindred' and 'Vantage' (Mohamed, Okiror, Rasmusson, *Crop Sci.* 18: 418-422; 1978)

Winter, 1992-93:

F<sub>1</sub> Plants grown in the greenhouse at Logan, Utah.

There was no segregation observed in  $F_1$  plants.

Summers, 1993, 1994 and 1995:

F<sub>2</sub> through F<sub>4</sub> generation plants grown in the field at Logan, Utah in space-planted (plants 6 inches apart with 12-inch row spacing) modified bulk populations which were selected for plants possessing the following characteristics:

- · Four or more fertile tillers per plant in space-planted stands
- · Early to mid-season heading date
- · Early to mid-season maturity date
- · Less than 90 cm tall
- · Zero to near-zero lodging
- · Upright stems
- · Desirable plant confirmation
- · Plump seeds
- · White aleurone
- · Complete exertion of spike from flag leaf at maturity
- · Tough (not brittle) stem and neck
- · Lemma awns longer than spike
- · Moderately free of powdery mildew (caused by Erisyphe graminis DC. F sp. Herdei Em. Marchal)
- · Aquila is susceptible to barley stripe rust (caused by Puccinia striiformis Westend)

Selected seed was bulked for each succeeding generation.

Summer, 1996:

F<sub>5</sub> Plants grown at Logan, Utah in a space planted (plants 6 inches apart with 12 inch row spacing) modified bulk population and single heads were selected from 200 plants possessing the same characteristics as those listed for the F<sub>2</sub> through F<sub>4</sub> generations.

Summer, 1997:

Seed from the 200 individual selected heads were grown in F6 head rows at Logan, Utah, where all rows were evaluated for the same characteristics as those listed for the F2 through F5 generations. Only desirable rows were harvested. Seed from harvested rows were subjected to protein evaluation and kernel rating in the lavatory. Row 1632 (identified as UT97B 1480-1632) was selected as a single head row for additional testing. It was found to breed true for rough lemma awns.

Summer, 1998 and 1999:

UT97B 1480-1632 was evaluated for yield and test weight, in addition to the characters listed for the  $F_6$  head rows, in a single-replicate preliminary irrigated yield test (which included Steptoe check plots) grown at Logan, Utah.

Summers, 2000 through 2003:

UT97B 1480-1632 was evaluated for the same characters listed for the preliminary irrigated yield test, in replicated irrigated yield tests at four major irrigated barley production sites in Utah.

Summers, 2001 Through 2003:

UT97B 1480-1632 was evaluated for the same characters listed for the preliminary irrigated yield test, in and the Western Regional Irrigated Spring Barley Nursery.

Summer 2002:

Selected 1000 heads of UT97B 1480-1632 were selected among the  $F_{5-11}$  progenies at Logan, Utah to be used for production of Breeder seed.

Winter, 2002-03:

Breeder seed of UT97B 1480-1632 was produced at Yuma,

Arizona, from the selected 1000 heads. The 1000 single head rows were rogued for off types, retained rows were harvested in bulk to

constitute the breeder seed.

Summer 2003: Foundation seed of Aquila (UT97B 1480-1632) was produced at

Cache Junction, Utah from Breeder seed produced in winter of

2003.

Summer 2004:

Registered seed of Aquila was produced.

Summer 2005:

Certified Seed of Aquila was produced to be marketed for commercial production.

Aquila has been observed to be stable for 6 generations (beginning with the  $F_6$  head row from which it originated in 1998, through the  $F_{11}$  Foundation field produced in 2003). There have been no variants observed. Any questionable plants rogued from Breeder and Foundation plantings were likely due to environmental variations. They were removed strictly as a precautionary measure.

# Exhibit B - Novelty Statement for Aquila

Aquila (a six-rowed type) mostly nearly resembles Millennium and Steptoe barleys. Differences between Aquila and the other two varieties include, but are not restricted to, the following characteristics:

- 1. Head shape of Aquila slightly tapered like Steptoe, while Millennium is a tapered head shape.
- 2. Head density of Aquila [Lax (Not dense), (2.9-3.1 mm/internode)] is similar to that of Millennium [Erect (Not dense), (2.4-2.7 mm/internode)], and more dense than Steptoe [Lax (3.2-3.5 mm/internode)].
- 3. Aquila has no overlapping of upper lateral spikelets, while Millennium and Steptoe has some overlap of lateral kernels at the tip of the head.
- 4. Aquila, Millennium, and Steptoe all have rachis edge covers with hairs.
- 5. Aquila, Millennium, and Steptoe all have glume awns longer than the glumes.
- 6. Aquila is covered with short hairs on the ventral surface of the glumes, while Millennium has more visible hairs on the ventral surface of the glumes than Aquila, and Steptoe is covered with hairs on the ventral surface of the glumes.
- 7. The lemma base shape of Aquila has a depression as in Millenium whereas Steptoe has a transverse crease.

On exhibit D, we present additional genotyping of Aquila with 32 barley microsatellites or SSR markers. We found polymorphism between Aquila vs Millenium or Steptoe for fourteen SSR markers covering all seven chromosomes of barley.

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0581-0055. The time required to complete this information collection is estimated to average 1.5 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

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To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 14th and Independence Avenue, SW, Washington, DC 20250-9410 or call 202-720-5964 (voice and TDD). USDA is an equal opportunity provider and employer.

U.S. DEPARTMENT OF AGRICULTURE AGRICULTURAL MARKETING SERVICE SCIENCE AND TECHNOLOGY PLANT VARIETY PROTECTION OFFICE BELTSVILLE, MD 20705

# OBJECTIVE DESCRIPTION OF VARIETY Barley (Hordeum vulgare L.)

		-			
NAME OF APPLICANT (S)	TEMPORARY O	R EXPERIMENTAL DESIG	ENATION	VARIETY NAME	
Utah Agricultural Experiment Station	UT97B148	0-1632		Aquila	
ADDRESS (Street and No. or RD No., City, State, Zip Code, and Utah State University 4820 Old Main Hill	Country)			FOR OFFICIAL USE ONLY  PVPO NUMBER  20060	n n 4 0
Logan, UT 84322				EUUUU	79 -8 -8
PLEASE READ ALL INSTRUCTIONS CARE	FULLY:				
Place the appropriate number that describes the when the number is either 99 or less or 9 or 10 or		f this variety in the bo	xes below. Place a	zero in the first box (i.e., 0 9	9 or 0 9 )
1. GROWTH HABIT:  1 = Spring 2 = Facultative Winter	r 3 = Winter	Early Growth:	3 1 = Prostra	ate 2 = Semi-Prostrate	3 = Erect
2. MATURITY: (50% Flowering)  2	2 = Mid-Season (Betze teptoe	es) 3 = Late (Frontie	r)		
Same as Check	//A //A	*			
3. PLANT: (From Soil Level to Top of Head)  3	California Mariout) 1	3 = Medium 1	all (Betzes)	4 = Tall (Conquest)	
4. STEM:  3 Exsertion (Flag to Spike at Maturi 2 Anthocyanin: 1 = Absent  05 No. of Nodes (Originating from Note) 4 Collar Shape: 1 = Closed  1 Shape of Neck: 1 = Straight	2 = Present	2 = (3 - 10 cm)  3 = Open  3 = Other (Specify)	3 = (10 – 15 cm) 4 = Modified Close	d or Open	

<sup>\*</sup> A commercial variety grown in the same trial.

5. LEAF: 200600040 1 Basal Leaf Sheath (Seedling): 1 = Glabrous 2 = Pubescent 2 Position of Flag Leaf (At Boot Stage): 1 = Drooping 2 = Upright 2 Waxiness: 1 = Absent (Glossy) 2 = Slightly Waxy 3 = Waxy 015 mm Width (First Leaf Below Flag Leaf) 17.5 cm Length (First Leaf Below Flag Leaf) Anthocyanin in Leaf Sheath: 1 = Absent 2 = Present 6. HEAD: 2 Type: 1 = Two-Rowed 2 = Six-Rowed 2 Density: 1 = Lax 2 = Erect (Not Dense) 3 = Erect (Dense) 4 = Other (Specity) \_\_\_\_\_ 10-5-2006 Shape: 1 = Tapering 2 Strap 3.= Clavate 4 = Other (Specify) Waxiness 1 = Absent (Glossy) 2 = Slightly Waxy Lateral Kernels Overlap: 1 = None 2 = At Tip 3 = 1/4 - 1/2 of Head Rachis (Hair on Edge): 1 = Lacking 2 = Few 3 = Covered 7. GLUME: 3 Length: 1 = 1/3 of Lemma 2 = 1/2 of Lemma 3 = More than 1/2 of Lemma 2 Hairs: 1 = None 2 = Short 3 = Long Hair Covering: 1 = None 2 = Restricted to Middle 3 = Confined to Band 4 = Completely Covered Awns: 1 = Less than Equal to Length of Glumes 2 = Equal to Length of Glumes 3 = More than Equal to Length of Glumes Awn Surface: 1 = Smooth 2 = Semi-Smooth 3 = Rough 8. LEMMA: 5 Awn: 1 = Awnless 2 = Awnlets on Central Rows, Awnless on Lateral Rows 3 = Short on Central Rows, Awnlets on Lateral Rows 4 = Short (Less than Equal to Length of Spike) 5 = Long (Longer than Spike) 6 = Hooded Awn Surface: 1 = Awnless 2 = Smooth 3 = Semi-Smooth 4 = Rough Teeth: 1 = Absent 2 = Few 3 = Numerous Hair: 1 = Absent 2 = Present

2 = Slight Crease

3 = Transverse Crease

#### 9. STIGMA:

Shape of Base:

Raachilla Hairs:

1 Hairs: 1 = Few 2 = Many

1 = Short

1 = Depression

2 = Long

10. SEED:					ğ	nanea	n n k n
2 Type: 1 =	Naked	2 = Covered			ģ.	20060	U U T U
1 Hairs on Ventral F	urrow:	1 = Absent	2 = Preser	nt			
2 = 3 = 4 =	Mid-long (8.5 -	ng (7.5 – 9.0 mm) - 9.5 mm) ng (9.0 – 10.5 mm)					
Wrinkling of Hull:	1 = Nake	•	/rinkled (	3 = Semi-Wrinkled	l 4 = Wrinkled		
1 Aleurone Color:	1 = Colo	rless (White or Yellov	w) 2	2 = Blue			
3 Percent Abortive			40 7	GMS. per 1000 Se	eeds		
11. DISEASE: (0 = Not Tested, 1	= Susceptible,	2 = Resistant, 3 = Int	termediate, 4	I - Tolerant)			
0 Septoria	Net Bloto	ch 0	Spot Blotch	0	Powdery Mildew		
3 Loose Smut	) Bacterial	Blight 0	Covered Si	mut 0	False Loose Smut		
0 Stem Rust	Leaf Rus	it 0	Scab	0	Scald		
0 Aster Yellows Virus	BSMV	0	BYDV		Other (Specify)	*****	<del></del>
12. INSECT: (0 = Not Tested, 1 =	Susceptible, 2	= Resistant, 3 = Inte	rmediate, 4	- Tolerant)			· · · · · · · · · · · · · · · · · · ·
O Green Bug	English (	Grain Aphid 0	Chinch Bug	0	Armyworm		
Grasshoppers	Cerial Le	af Beetle	Other (Spe	cify)			<u></u>
Hessian Fly Races	0 <sub>GP</sub>	0 A		0 В 0 <sub>F</sub>	0 c	Other Specify)	· · · · · · · · · · · · · · · · · · ·
13. CHEMICAL: (0 = Not Tested, 1 = Susceptible, 2 = Resistant, 3 = Intermediate, 4 = Tolerant)  Other (Specify)							
14. INDICATE WHICH VAREITY I	MOST CLOSE	Y RESEMBLES TH	AT SUBMIT	TED:		· <del>[····</del>	
CHARACTER		NAME OF VARIE	TY	CI	HARACTER	NAME O	F VARIETY
Plant Tillering	Miller	nnium		Seed Size		Baronesse	noni
Leaf Size	Stept	oe		Coleoptile Elo	ngation	Steptoe	· · · · ·
Leaf Color	Miller	nnium		Seedling Pigm		Steptoe	
Leaf Carriage	Step	oe	-				<del></del>

### REFERENCES:

The following publications may be used as a reference aid for the standardization of character descriptions and terms used in this form:

- Wiebe, G.A., and D.A. Reid, 1961, Classifications of Barley Varieties Grown in the United States and Canada in 1958, Technical Bulletin No. 1224, U.S. Department of Agriculture.
- 2. Reid, D.A., and G.A. Wiebe, 1968, Barley: Origin, Botany, Culture, Winter Hardiness, Genetics, Utilization, Pests, Agriculture Handbook No. 338, U.S. Department of Agriculture, pp. 61-84.
- 3. Malting Barley Improvement Association, Milwaukee, Wisconsin, 1971, Barley Variety Dictionary.

COLOR: Nickerson's or any recognized color fan may be used to determine color of the described variety.

# Exhibit D: Additional genotyping of Aquila barley

# **Materials and Methods**

These molecular experiments were conducted by Dr. Shiaoman Chao at the USDA-ARS Biosciences Research Lab in Fargo (SD). In these experiments we surveyed a set of 32 barley microsatellites or SSR markers (Ramsay, et al., 2000). We will give results for fourteen of these SSR markers that were found to be polymorphic between Aquila and Millennium and Steptoe barley genotypes. Their respective sequences are presented in Table I. We employed fluorescent-based genotyping technology using a semi-automated capillary gel system, ABI3130xl, from Applied Biosystems.

#### PCR reactions

The PCR reaction setup was based on the M13-tailed PCR method (Boutin-Ganache, et al, 2001) after optimization. The forward primers were modified by adding 19 bases of M13 derived sequence to their 5'end. The 19-base M13 primer was labeled with one of the four fluorescent dyes, FAM, VIC, NED and PET. For PCR reactions, 50ng of DNA template was used along with a modified forward primer, reverse primer and M13 primer labeled with one dye added at a molar ratio of 0.15:1:1. The total reaction volume was 10 microliters. The cycling condition used was based on published results for particular SSRs (Ramsay, et al., 2000).

# Gel Electrophoresis and fragment analysis

The PCR products labeled with four different fluorescent dyes were pooled. The pooled samples were subjected to gel electrophoresis after adding the size standards. The gel electrophoresis was carried out on the ABI3130xl sequencer, a 16-capillary gel system. Fragment sizing and allele calling were performed using the GeneMapper v3.7 software from Applied Biosystems. The fragment size call is based on the Local Southern algorithm.

#### Results

We present fluorescent profiles for Aquila, Millennium and Steptoe that were generated with fourteen sets of SSR-primers (Figures 1-14). We found polymorphism for all seven chromosomes of barley.

On chromosome 1 (chr 1), with the <u>WMC1E8</u> marker (Fig. 1), we found DNA fragment sizes of 253 base pairs (bp) for Aquila, and 208 bp for Millennium and Steptoe. On chr 2, all three lines differ in fragment sizes generated with marker <u>Bmac0134</u> (Fig. 2) (142 bp for Aquila, 139 bp for Millennium, 166 bp for Steptoe) and <u>Bmag0125</u> (Fig.3) (154 bp for Aquila, 160 bp for Millennium, 152 bp for Steptoe).

On chr 3, we found polymorphism with four SSR markers. In all four cases the allele found in Aquila was different from the one shared by Millennium and Steptoe; for

Bmag0136 (Fig. 4), 220 bp vs 218 bp, for Bmac0209 (Fig.5), 206 vs 195 bp, for Bmag0013 (Fig.6), 179 vs 173 bp, and for Bmag0877 (Fig.7), 169 vs 165 bp. On chr 4, we found polymorphism for three SSR markers. For Bmac0310 (Fig.8), the allele size is 192 bp for Aquila and 159 bp for both Millennium and Steptoe. For EBmac0701 (Fig.9), the allele size of Aquila (166bp) differs from those of Millennium (160 bp) and Steptoe (152 bp). Finally, for EBmac0788 (Fig.10), Aquila has an allele size of 192 bp as Millennium and Steptoe have allele sizes of 185 and 178 bp, respectively. On chr 5, for the Bmag0337 marker (Fig. 11), the allele size for Aquila is 163 bp as that of Millennium and Steptoe is 167 bp.

On chr 6, we found two polymorphic SSR markers. For <u>Bmag0496</u> (Fig. 12), the allele size for Aquila is 213 bp as those of Millennium and Steptoe are 209 and 205 bp, respectively. For <u>EBmac0806</u> (Fig. 13), we found an identical allele size for Millennium and Steptoe (178 bp) as that of Aquila was slightly larger (186 bp).

Finally on chr 7, for the <u>Bmag0120</u> marker (Fig. 14), we found that the allele for Aquila was slightly smaller than that shared by Millennium and Steptoe (248 vs 250 bp)

## References

Boutin-Ganache, I., M. Raposo, M. Raymond and C.F. Deschepper (2001). M13-tailed primers improve the readability and usability of microsatellite analysis performed with two different allele-sizing methods. *BioTechniques* 31(1):25-28.

Ramsay, L., M. Macaulay, S. degli Ivanissevich, K. MacLean, L. Cradle, J. Fuller, K.J. Edwards, S. Tuvesson, M. Morgante, A. Massari, E. Maestri, N. Marmiroli, T. Sjakste, M. Ganal, W. Powell and R. Waugh (2002). A simple sequence repeat-based linkage map of barley. *Genetics* 156:1997-2005.

 Table 1: Microsatellite markers utilized in the genotyping of Aquila, Millenium and Steptoe barley lines.

 SSR abbrevations, chromosome assignment, genetic map location and sequences for forward and reverse primers are indicated.

Reverse	TCAATGCCCTTGTTTCTGACCT	CTTCGTTGCTTCTACCTT AGATAACGATGCACCACC	TAGTTTTCCCAAAAGCTTCTA ATGCCTGTGTGTGGACCAT TCGAATAGGTCTCCGAAGAAA GTAGGAGGAAGAATAAGGAGG	ATCTAGTGTGTTTGCTTCCT TGGCACTAAAGCAAAAGAC ATGATGAGAACTCTTCACCC	GACCCATGATATGAAGATCA	CTATAGCACGCCTTTGAGA GTGTGTAGTAGGTGGGTACTTG	GTCACATAGACAGTTGTCTTCC
Forward	TCATTCGTTGCAGATACACCAC	CCAACTGAGTCGATCTCG AATTAGCGAGAACAAAATCAC	AAAGCTCATGGTAGATCAAGA CTAGCAACTTCCCAACCGAC AAGGGGAATCAAAATGGGAG GTACGCTTTCAAACCTGG	CTACCTCTGAGATATCATGCC ATGATGAGAACTCTTCACCC TAACTTATATATCCATGGCA	ACAAAGAGGAGTAGTACGC	AGTATAACCAACAGCCGTCTA ACTAAGTCCTTTCACGAGGA	ATTTCATCCCAAAGGAGAC
Repeat motif	(AC)24	(AC)28 (AG)19	(GA)15 (AC)13 (CT)21 (AG)6-(AG)10-(AG)6	(CT)11(AC)20 (AC)23 (TG)23	(AG)22	(CT)20 (CA)4GA(CA)8,(CA)5	(AG)15
Map location (cM)	164	5	141 55 141 50	43 76 90	35	96 119	118
chr.	↔	2 2	мммм	4 4 4	ſŲ	9 9	7
SSR	WMC1E8	Bmac0134 Bmag0125	Bmag0877 Bmac0209 Bmag0013 Bmag0136	Bmac0310 EBmac0701 EBmac0788	Bmag0337	Bmag0496 EBmac0806	Bmag0120

Figure 1: Results of gel electrophoresis and fragment analysis with SSR marker WMC1E8 for Aquila, Millenium and Steptoe barley genotypes. 8 ş 8 8 4 Z. NA NA NA 4 Remortéd s1724 **4** 50 50 WATCHES & WMC1E8 WARCIES ( dom-4 **F** dom-4 Millenium Aquila Steptoe 010\_E06 fea A4 014\_C06 Fst | NA 016\_H06.64 S4 1600 1200 180 1400

13

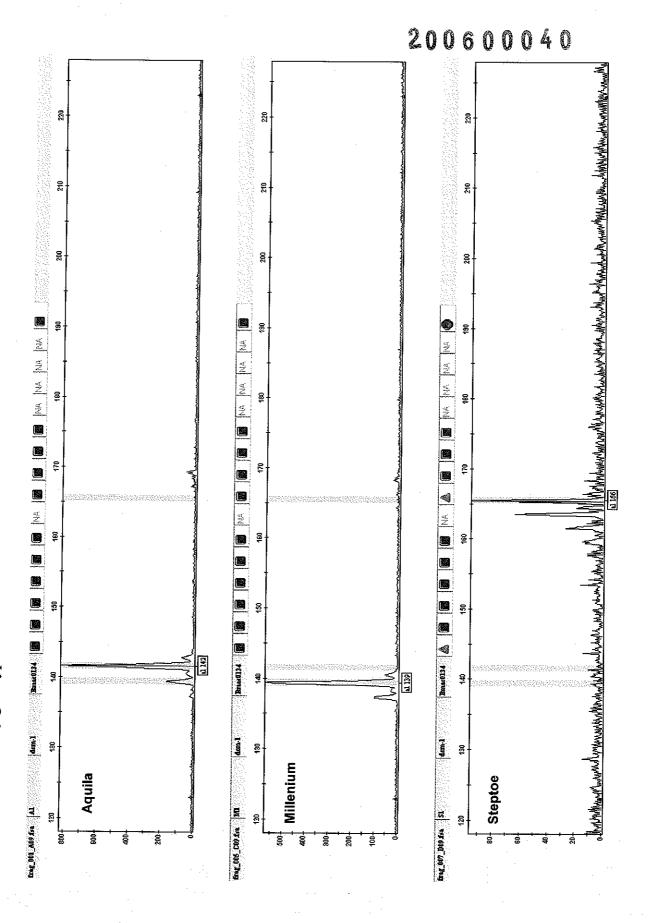


Figure 3: Results of gel electrophoresis and fragment analysis with SSR marker Bmag0125 for Aquila, Millenium and Steptoe barley genotypes.

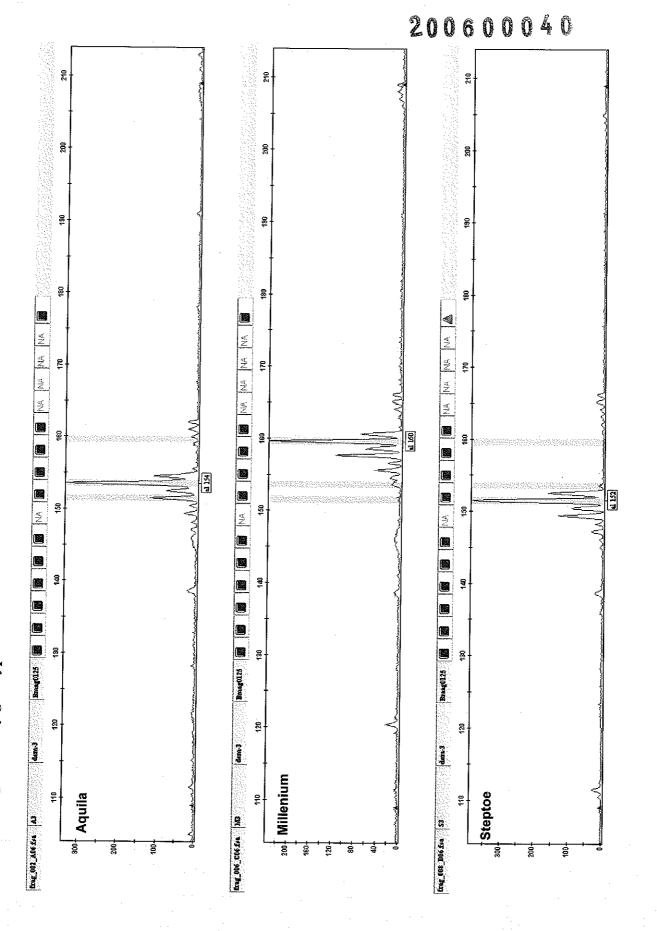


Figure 4: Results of gel electrophoresis and fragment analysis with SSR marker Bmag0136 for Aquila, Millenium and Steptoe barley genotypes.

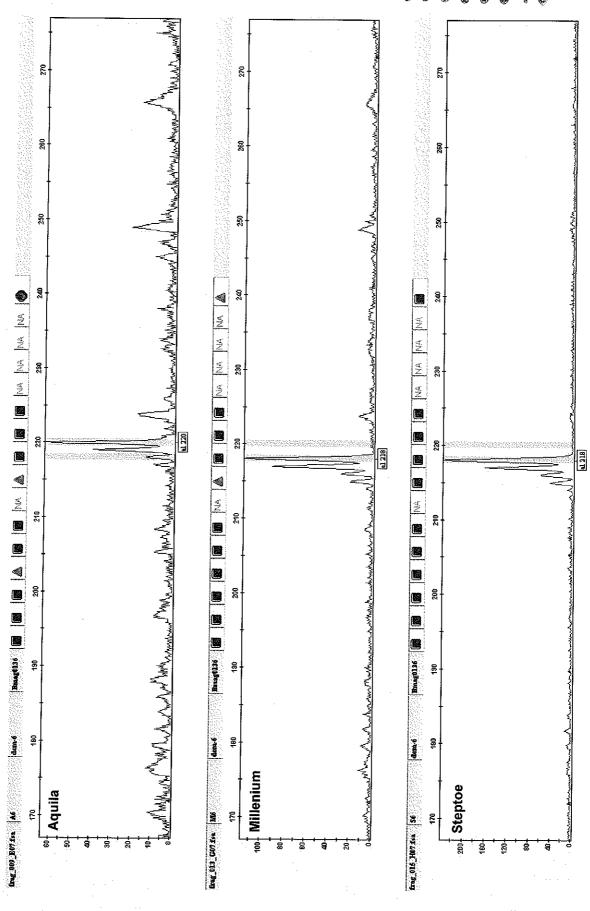


Figure 5: Results of gel electrophoresis and fragment analysis with SSR marker Bmac0209 for Aquila, Millenium and Steptoe barley genotypes.

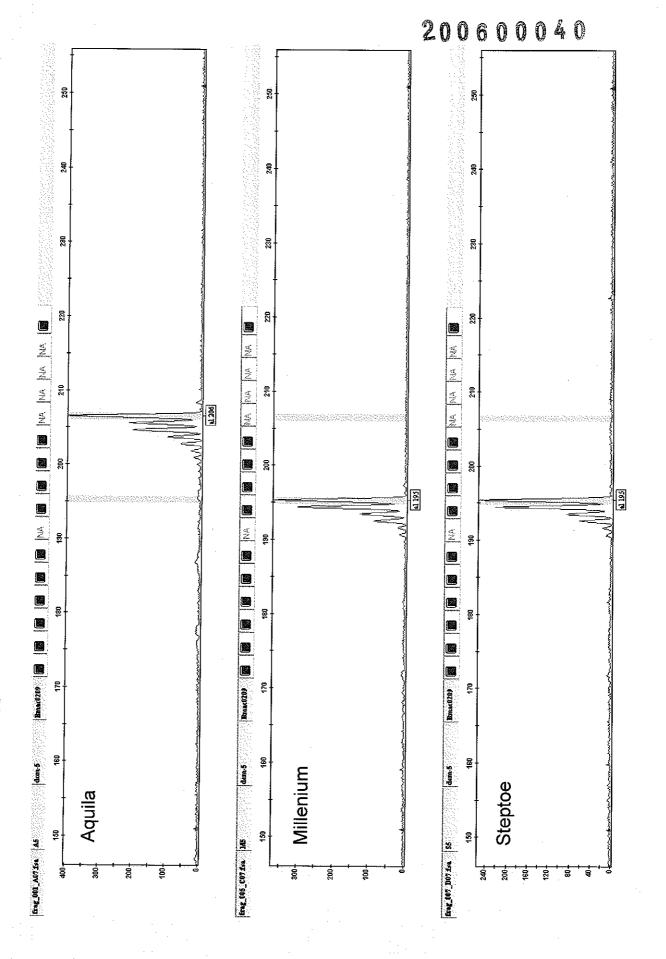


Figure 6: Results of gel electrophoresis and fragment analysis with SSR marker Bmag0013 for Aquila, Millenium and Steptoe barley genotypes.

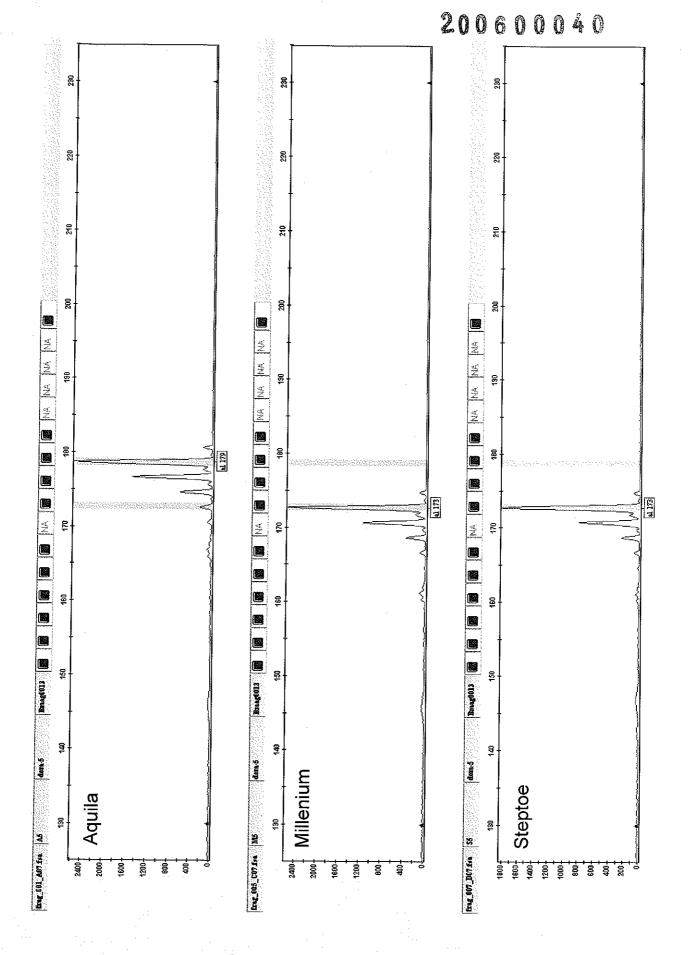


Figure 7: Results of gel electrophoresis and fragment analysis with SSR marker Bmag0877 for Aquila, Millenium and Steptoe barley genotypes.

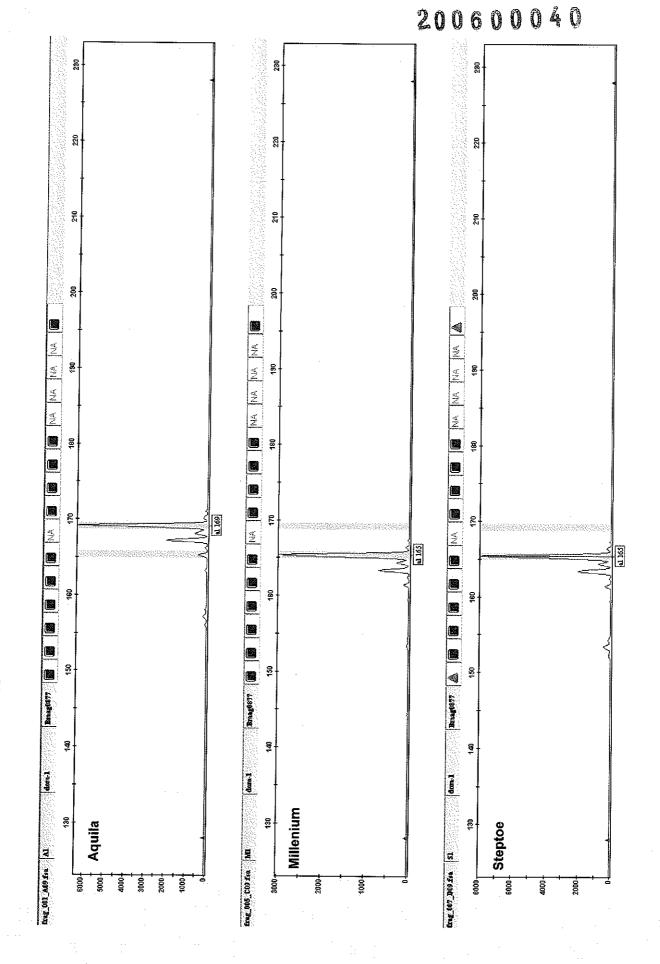


Figure 8: Results of gel electrophoresis and fragment analysis with SSR marker Bmac0310 for Aquila, Millenium and Steptoe barley genotypes.

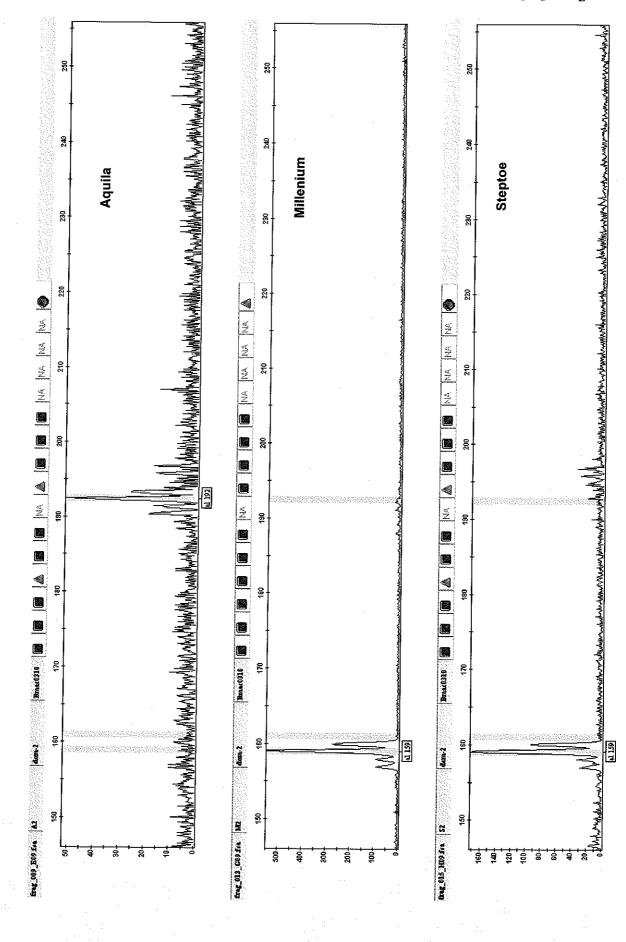


Figure 9: Results of gel electrophoresis and fragment analysis with SSR marker EBmac0701 for Aquila, Millenium and Steptoe barley genotypes.

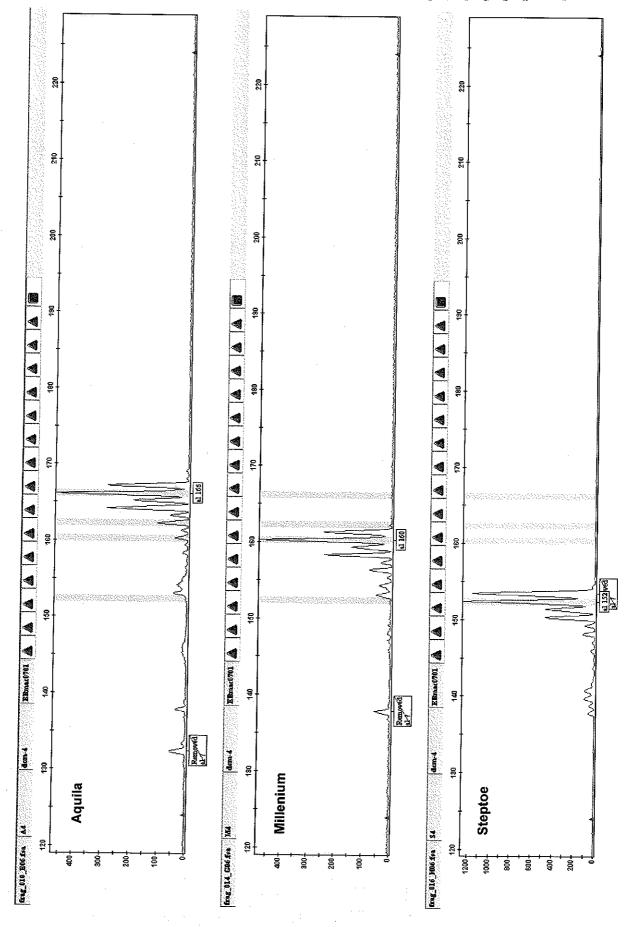


Figure 10: Results of gel electrophoresis and fragment analysis with SSR marker EBmac0788 for Aquila, Millenium and Steptoe barley genotypes.

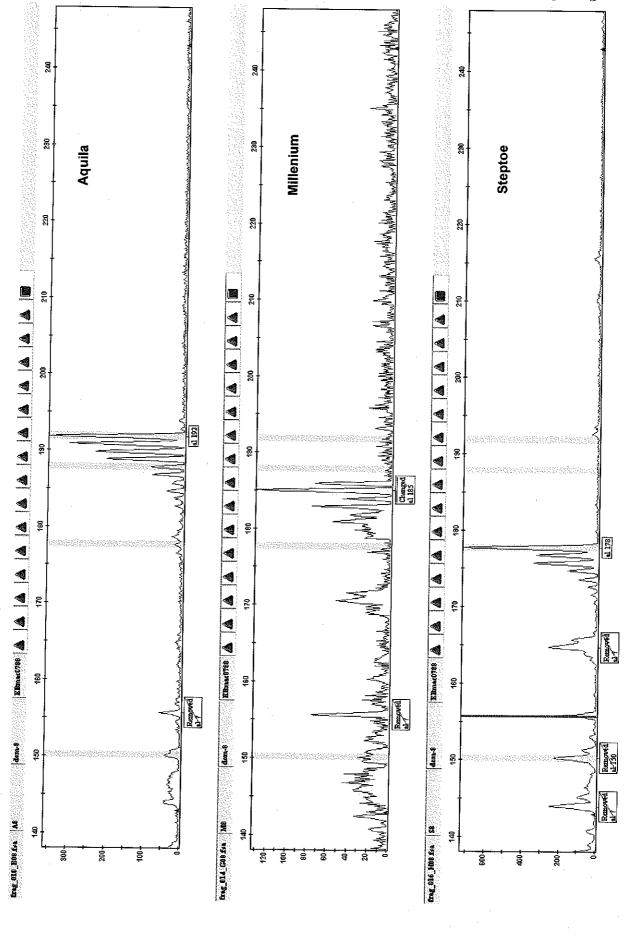


Figure 11: Results of gel electrophoresis and fragment analysis with SSR marker Bmag0337 for Aquila, Millenium and Steptoe barley genotypes.

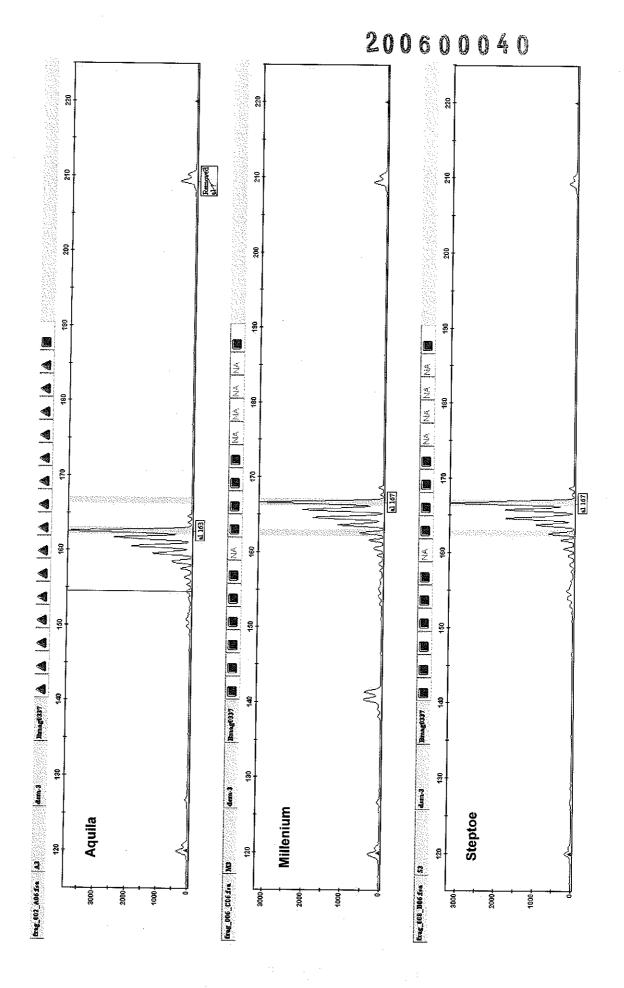
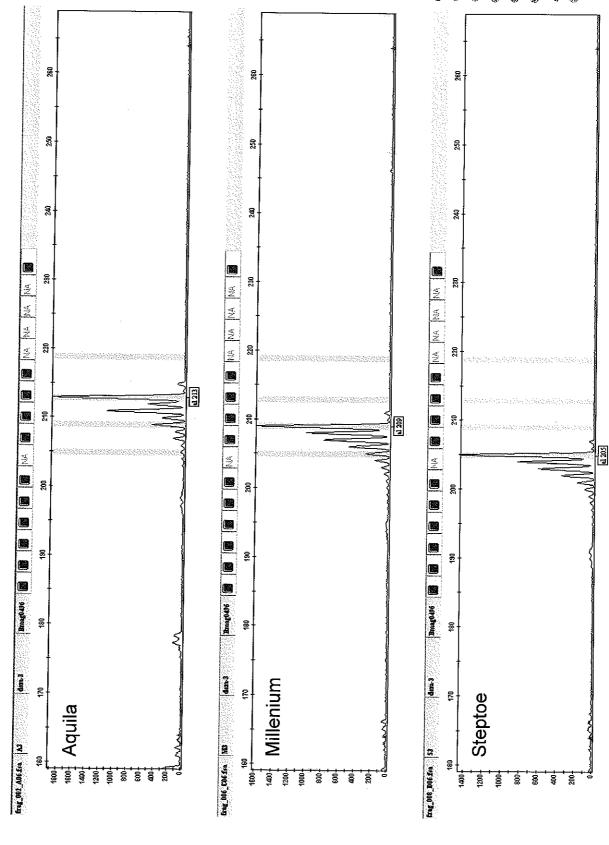


Figure 12: Results of gel electrophoresis and fragment analysis with SSR marker Bmag0496 for Aquila, Millenium and Steptoe barley genotypes.



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Figure 13: Results of gel electrophoresis and fragment analysis with SSR marker EBmac0806 for Aquila, Millenium and Steptoe barley genotypes.

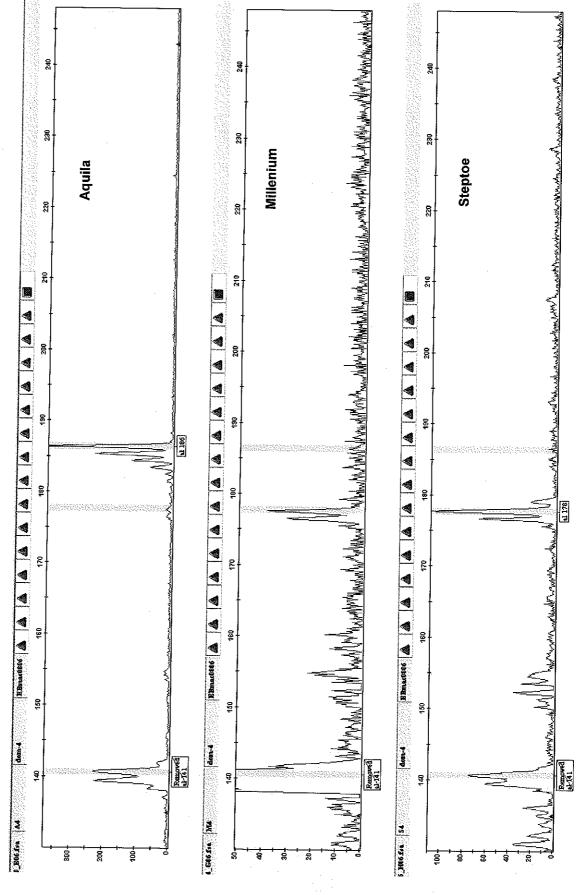
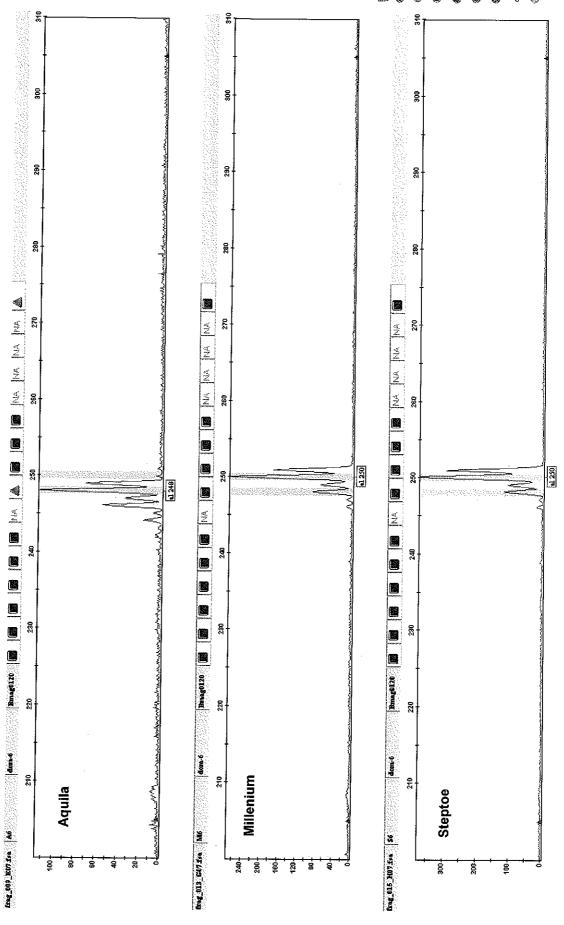


Figure 14: Results of gel electrophoresis and fragment analysis with SSR marker Bmag0120 for Aquila, Millenium and Steptoe barley genotypes.



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	Logan, UT 84322	VARIETY NAME Aquila		
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Dr. Dominique Roche	4820 Old Main Hill Logan, UT 84322	PVPO NUMBER		
		200600040		

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Signature